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Effect of Stimulation of the Host Defense System* by Coenzyme Q_{10} on Dibenzpyrene-Induced Tumors and Infection with Friend Leukemia Virus in Mice

(neoplasia/reticuloendothelial system)

EMILE G. BLIZNAKOV

New England Institute, Ridgefield, Connecticut 06877

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ABSTRACT **Members of the coenzyme Q group increase the phagocytic activity in rats, as measured by the carbon clearance technique, and increase the hemolytic antibody formation in mice. In addition, prior treatment with low doses of chloroquine hydrochloride combined with coenzyme Q_{10} results in increased numbers of survivors, prolonged survival time, and reduced parasitemia in blood-transferred *Plasmodium berghei* infection in mice**

In an extension of these studies, using emulsions of coenzyme Q_{10}, I demonstrated the following effects on two tumor systems in mice: (*i*) Treatment with coenzyme Q_{10} decreased splenomegaly and hepatomegaly and increased the number of surviving mice infected with Friend leukemia virus. (*ii*) Treatment with coenzyme Q_{10} reduced the percentage of mice with tumors, increased the number of survivors, and reduced the tumor size in mice with tumors induced by 3,4,9,10-dibenzpyrene. The effect on both tumor systems was dose-dependent. These studies support the hypothesis that the host defense system plays a definitive role in the defense of the host against invasion by various agents, including neoplasia.

The importance of the host defense system [which includes, in part, the reticuloendothelial system (RES)] in resistance to neoplasia has been demonstrated clinically and in experimental animals and has been the subject of studies by a growing number of investigators (1–12). Substances known to stimulate the RES, such as bacterial endotoxin, zymosan, glucan, Bacillus Calmette-Guérin, and others, inhibit the growth of some experimental tumors (1, 2, 4, 5, 10, 13–19). In contrast, substances that depress RES activity stimulate the growth of some tumors or increase the incidence of metastasis in cases in which neoplasm already exists (10, 20–24). None of the attempts at stimulation of the host defense has, by itself, given entirely satisfactory or practical results (25). Furthermore, virtually all agents used thus far are more or less toxic and cause histological changes, including gross organ hyperplasia.

In this Institute we have maintained an extensive search for nontoxic stimulants of the host defense system since 1954. We demonstrated that lipidic fractions derived from fresh shark livers† (*Negaprion brevirostris*) produce host defense stimulation in various experimental models without producing side effects (27–35). Based on the information that shark livers contain coenzymes Q (36, 37), biological evaluation of pure, commercially available coenzymes Q demonstrated that coenzymes Q_6 and Q_{10} (Fig. 1) increase the phagocytic activity in rats, as measured by the carbon clearance technique, and increase primary hemolytic antibody formation in mice (38, 39). Furthermore, prior treatment with coenzyme Q_{10} combined with low doses of chloroquine hydrochloride resulted in increased numbers of survivors, prolonged survival time, and reduced parasitemia in blood-transferred *Plasmodium berghei* infection in mice (35, 40).

Structure–activity relationship studies suggested that coenzyme Q_{10} produces a more pronounced effect than the other coenzymes Q tested and that there is a structural requirement in the coenzyme Q family for stimulation of phagocytic activity for a quinoidal moiety with at least a partially unsaturated side chain (39). In addition, coenzymes Q_6 and Q_{10} showed no pyrogenic effect in rabbits, according to U.S. Pharmacopeia-recommended criteria (41), and they induce no significant proliferative effect on the RES (35, 38–40). None of the toxicological studies, including our own, of various coenzymes Q revealed any significant abnormalities that would contraindicate their use in humans (42–44).

In the present study I attempted to establish the significance of enhanced host defense activity produced by coenzyme Q_{10} on resistance of mice to tumors induced by 3,4,9,10-dibenzpyrene and infection with Friend leukemia virus (FLV).

MATERIALS AND METHODS

Animals. Male Swiss Webster (Blue Spruce Farms, Inc., Altamont, N.Y.) or $C_{57}Bl_6$ (Jackson Laboratory, Bar Harbor, Me.) mice, weighing 20 g, were obtained from a single commercial breeder. They were housed in air-conditioned rooms artificially illuminated on controlled cycles during daylight hours and kept at a uniform humidity with a temperature of 72 ± 2°F. Food and water were freely available.

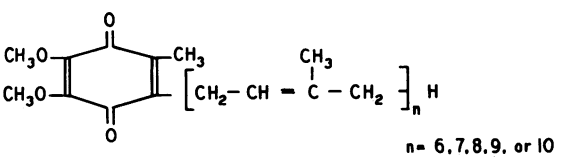

FIG. 1. Coenzyme Q or ubiquinone.

Abbreviations: FLV, Friend leukemia virus; RES, reticuloendothelial system.

* The term "host defense system" is taken in this paper as the broadest definition of this system.

† In subsequent studies coenzymes Q were identified as components of these extracts (26) that are apparently the active material.

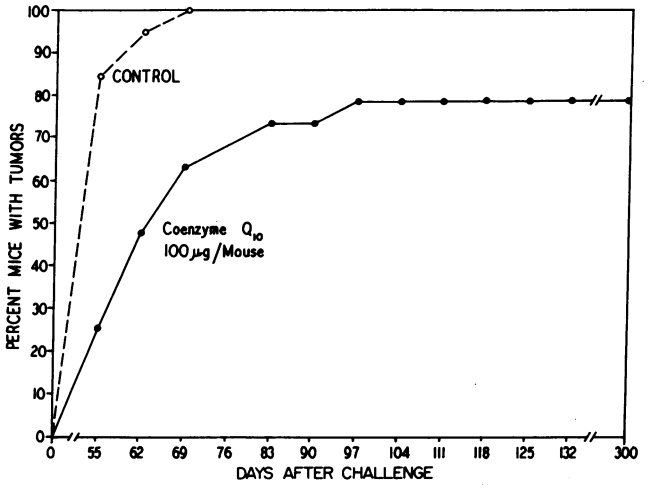

FIG. 2. Modification of the percentage of animals with tumors induced by dibenzpyrene in mice treated with coenzyme Q_{10}.

Coenzyme Q_{10}. Commercially available coenzyme Q_{10} (Nutritional Biochemicals, Cleveland, Ohio) was used. Only pure coenzyme Q_{10} was used in these studies; the degree of purity was evaluated as described (39).

Coenzyme Q_{10} was injected into the tail vein as an emulsion in 5% glucose solution. As an emulsifier Ninol (lauric diethanolamide) or Tween 20 (polyoxyethylene sorbital monolaurate) was used. The emulsions were prepared in a 500-ml Waring blendor, kept in a water bath at 60°C, and protected from light. Initial homogenization was 45 sec. The smaller doses were injected first and the emulsion was again homogenized for 15 sec before the next larger dose was injected. The method used to prepare the emulsion and the subsequent handling are of critical importance. The particle size of the emulsions was under 2–5 µm.‡

The control animals were injected with the same mixture, with coenzyme Q_{10} omitted.

Dibenzpyrene-Induced Tumors. Dibenzpyrene (Sigma Chemical Co., St. Louis, Mo.) was dissolved in peanut oil (Matheson Coleman and Bell, Cincinnati, Ohio). 0.5 ml was injected subcutaneously into the lateral abdominal wall of $C_{57}Bl_6$ mice. The concentration of the dibenzpyrene was 2 mg/ml. In preliminary experiments it was established that this dose produced local tumors in 100% of injected animals, and mortality higher than 90%, within 18–20 weeks after administration.

Coenzyme Q_{10} emulsions were prepared in 5% glucose containing 12.5 µg/ml of Ninol in a concentration of 500 µg/ml. Various amounts were administered intravenously 48 hr before and 7, 14, and 21 days after administration of dibenzpyrene.

The following parameters were studied: (*i*) percent of mice developing tumors; (*ii*) changes in tumor size in mice that developed tumors; and (*iii*) percent mortality.

Friend Leukemia Virus. A large batch of FLV was prepared as recommended by Rowe and Brodsky (45) and stored at −60°C. Preliminary tests established that 0.2 ml of this preparation, diluted 1:100 and administered intravenously into Swiss Webster mice, produced splenomegaly (mean spleen weight more than 1000 mg 20 days after infection) and mortality higher than 90%.

Coenzyme Q_{10} emulsions were prepared in 5% glucose containing 0.4% Tween 20 in a concentration of 250 µg/ml. Various amounts were administered intravenously 7, 14, 21, and 28 days after infection with FLV.

The following parameters were studied: (*i*) changes in body weight 30 days after infection; (*ii*) changes in spleen and liver weight 30 days after infection; (*iii*) ratio between liver and spleen weight and body weight 30 days after infection; and (*iv*) percent mortality 7 weeks after infection.

General. All glassware was heated for 5 hr at 170°C. Nonpyrogenic Ninol (Stepan Chemical Co., Nordfield, Ill.), Tween 20 (Sigma Chemical Co., St. Louis, Mo.), and sterile 5% glucose (Baxter Laboratories, Morton Grove, Ill.) were used throughout.

Possible contamination with bacterial endotoxin of the compounds used for preparation of emulsions was precluded by the use only of materials tested for pyrogenicity. Only nonpyrogenic materials were used in these studies. Criteria recommended by the U.S. Pharmacopeia were used for evaluation.

In each experiment a minimum of 20 mice per group was used at each dose. Further, each experiment was conducted at least twice. The data presented show the results of one of each set of duplicate experiments.

The data obtained were statistically analyzed by the chi-square test or Student's *t*-test, as applicable.

RESULTS

Coenzyme Q_{10} treatment administered 48 hr before and 7, 14, and 21 dyas after administration of the chemical carcinogen produced the following effects on dibenzpyrene-induced tumors in $C_{57}Bl_6$ mice: (*i*) reduction of the percentage of mice with tumors (Fig. 2). (*ii*) increase in number of survivors (Fig. 3). (*iii*) reduction of the tumor size of the mice that developed tumors during the observation period (Fig. 4). (*iv*) the effects were dose dependent (Fig. 5).

Coenzyme Q_{10} treatment administered 7, 14, 21, and 28

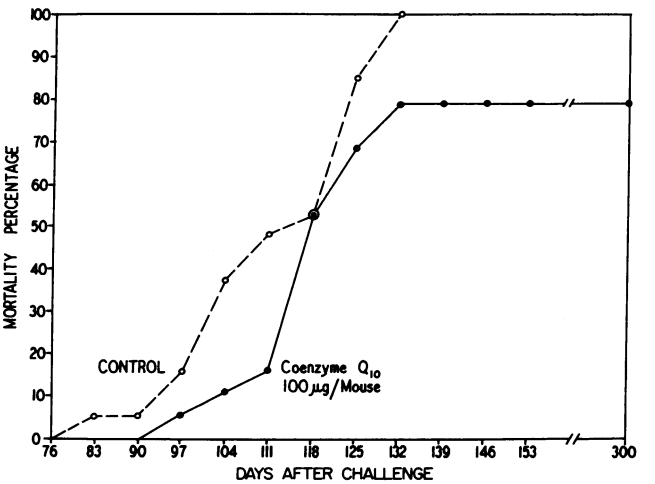

FIG. 3. Modification of the mortality in mice with dibenzpyrene-induced tumors and treated with coenzyme Q_{10}.

‡ Emulsions of coenzyme Q_{10} are also effective when injected subcutaneously (40). When coenzyme Q_{10} was "solubilized" in Emulphor EL 620 (42), no biological effect was observed at the doses tested.

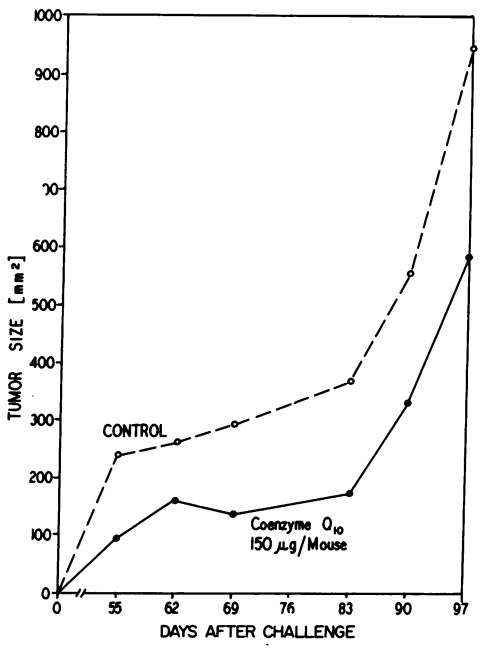

FIG. 4. Modification of the tumor size (mm^2) in mice with dibenzpyrene-induced tumors and treated with coenzyme Q_{10}. The calculations for tumor size are based only on the mice which developed tumors during the observation period.

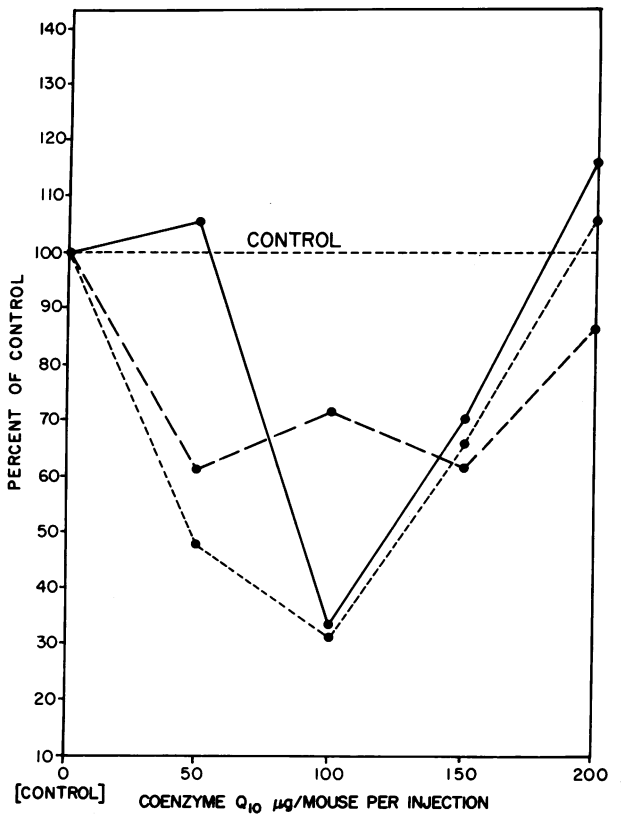

FIG. 5. Modification of the number of mice with tumors (day 55) (●- - -●), tumor size (mm^2, day 97) (●— —●), and mortality (day 111) (●——●) (all percent of control) in mice with dibenzpyrene-induced tumors and treated with various doses of coenzyme Q_{10}. Calculations for tumor size are based only on the mice that developed tumors during the observation period.

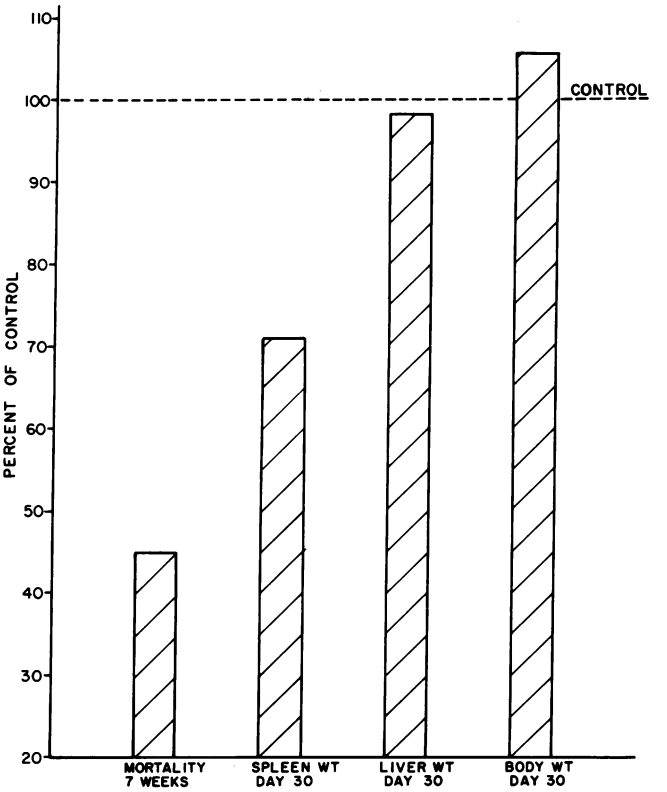

FIG. 6. Modification of spleen, liver, and body weight at 30 days and mortality at 7 weeks (all percent of control) in mice infected with Friend leukemia virus and treated with coenzyme Q_{10} (100 µg per mouse per injection). Body weight: without spleen and liver.

days after infection produced the following effects on FLV-induced leukemia in Swiss Webster mice (Figs. 6 and 7): (*i*) Decrease of splenomegaly and hepatomegaly 30 days after infection. (*ii*) Increase in number of survivors 7 weeks after infection. (*iii*) The effects were dose dependent.

Statistical analysis showed that the results and the conclusions obtained are all significant and that the differences observed are well outside the statistical limits of error. A Student's *t*-test of significance gives a *P* value ranging between 0.01 and 0.001.

DISCUSSION

The results reported here show that resistance to dibenzpyrene-induced tumors and leukemia resulting from infection with FLV in mice is significantly enhanced by the use of a nontoxic agent that stimulates host defense—coenzyme Q_{10}. The described effects are dependent on the dose of the coenzyme Q_{10} administered. This nonlinear, bimodal dose–effect relationship is not a property of the particular type of stimulant used (coenzyme Q_{10} in this case), but is characteristic of the entire host defense. Our detailed studies, as well as review of data published by others, support this view (35).

In recent years it has become increasingly obvious that two main types of responses are involved in host resistance to neoplasia (reviewed by Biozzi, ref. 46): nonspecific response essentially based upon stimulation of the macrophage system and specific immunological response against the tumor antigen(s) at the level of the lymphoplasmocytic cells. These two mechanisms are functionally interdependent, and their

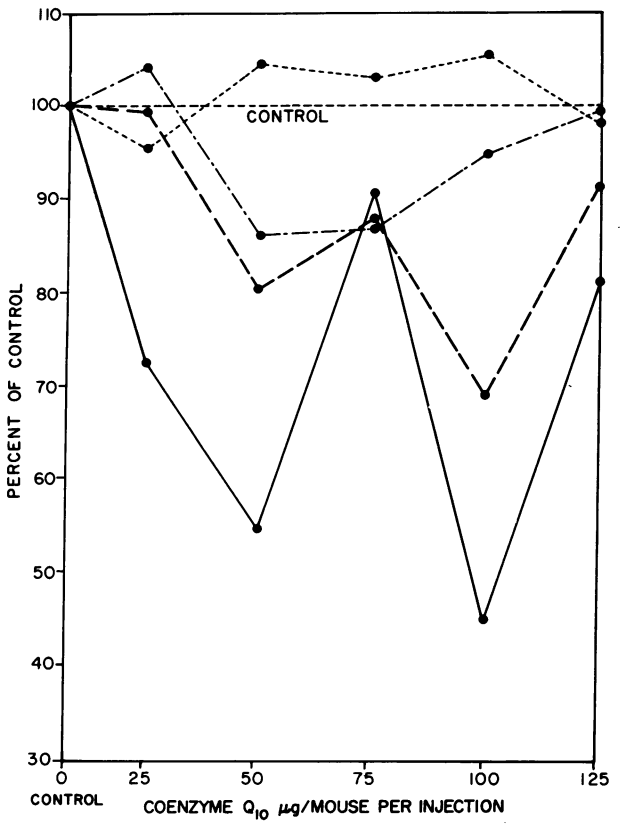

FIG. 7. Modification of body weight (●- - -●), (spleen weight/body weight) (●— —●), and (liver weight)/body weight (●-·-●) at 30 days and mortality (●——●) at 7 weeks (all percent of control) in mice infected with Friend leukemia virus and treated with various doses of coenzyme Q_{10}. Body weight: without spleen and liver.

stimulation leads to an increase in resistance to the occurrence and development of tumors.

Ubiquinones or coenzymes Q (Fig. 1) are an important family of lipid-soluble benzoquinones that play a major role in the electron transport systems of respiration and photosynthesis (reviewed by Redfearn, ref. 47). Despite recent advances, our knowledge of these processes is still far from complete, and it is not surprising that we do not yet understand the precise function of coenzymes Q. Therefore, any attempt to speculate on the mechanism of stimulation of host defense by coenzymes Q is premature. The possible parameters affecting neoplasia may include: (*i*) enhanced phagocytic capacity of the RES; (*ii*) increased ability of RES cells to destroy captured organisms intracellularly; (*iii*) presence of serum factor(s) increasing phagocytosis and intracellular destruction; (*iv*) restoration of the immunosuppression described as occurring in both tumor systems; (*v*) presence of stimulated immunologically activated cells; (*vi*) presence of "nonspecific" factors, e.g., interferon; and (*vii*) modification of the regulatory metabolic mechanism(s) of the host.

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